Transvection in the Drosophila *Ultrabithorax* Gene: A *Cbx*^{1} Mutant Allele Induces Ectopic Expression of a Normal Allele in *Trans*

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ABSTRACT

In wild-type *Drosophila melanogaster* larvae, the *Ultrabithorax* (*Ubx*) gene is expressed in the haltere imaginal discs but not in the majority of cells of the wing imaginal discs. Ectopic expression of the *Ubx* gene in wing discs can be elicited by the presence of *Contrabithorax* (*Cbx*) gain-of-function alleles of the *Ubx* gene or by loss-of-function mutations in *Polycomb* (*Pc*) or in other *trans*-regulatory genes which behave as repressors of *Ubx* gene activity. Several *Ubx* loss-of-function alleles cause the absence of detectable Ubx proteins (UBX) or the presence of truncated UBX lacking the homeodomain. We have compared adult wing phenotypes with larval wing disc UBX patterns in genotypes involving double mutant chromosomes carrying in *cis* one of those *Ubx* mutations and the *Cbx*^{1} mutation. We show that such double mutant genes are (1) active in the same cells in which the single mutant *Cbx*^{1} is expressed, although they are unable to yield functional proteins, and (2) able to induce ectopic expression of a normal homologous *Ubx* allele in a part of the cells in which the single mutant *Cbx*^{1} is active. That induction is conditional upon pairing of the homologous chromosomes (the phenomenon known as transvection), and it is not mediated by UBX. Depletion of *Pc* gene products by *Pc*^{3} mutation strongly enhances the induction phenomenon, as shown by (1) the increase of the number of wing disc cells in which induction of the homologous allele is detectable, and (2) the induction of not only a paired normal allele but also an unpaired one.

A considerable amount of genetic, developmental and molecular information is now available on genes of the bithorax complex, genes which control the metameric identity of some thoracic and all the abdominal segments of Drosophila (LEWIS 1978; reviewed in MORATA, SÁNCHEZ-HERRERO and CASANOVA 1986; DUNCAN 1987; PEIFER, KARCH and BENDER 1987; MAHAFFEY and KAUFMAN 1988; SÁNCHEZ-HERRERO, CASANOVA and MORATA 1988). One of those genes is *Ultrabithorax* (*Ubx*), which is responsible for the segmental identities of the anterior and posterior compartments of the third thoracic segment (T3a and T3p) and the anterior compartment of the first abdominal segment (A1a). The gene is also active in some cells of the posterior compartment of the second thoracic segment (T2p). The realm of action of the *Ubx* gene has been inferred from the phenotypes of its mutants in larval and adult cuticule (LEWIS 1978) and corroborated by means of RNA *in situ* hybridization (AKAM 1983; AKAM and MARTÍNEZ-ARIAS 1985) and by immunofluorescent staining of Ubx proteins (UBX) using the FP3.38 antibody, an antibody thought to recognize all the different UBX obtained by differential splicing (WHITE and WILCOX 1984).

The continuous activity of the *Ubx* gene is required during the development of T3 imaginal discs to maintain their proper morphogenetic identity (MORATA and GARCÍA-BELLIDO 1976). Two groups of *trans*-regulatory genes are known to be needed to maintain the correct spatial pattern of *Ubx* gene activity during development: (1) those of the *Regulator of bithorax* (also called *trithorax*) gene group, supposed to be necessary for the positive control of *Ubx* gene activity (INGHAM 1984, 1985a,b; INGHAM and WHITTLE 1980; CAPDEVILA and GARCÍA-BELLIDO 1981; SHEARN 1989), and (2) those of the *Polycomb* (*Pc*) gene group, supposed to be repressors (LEWIS 1978; DUNCAN and LEWIS 1982; CAPDEVILA, BOTAS and GARCÍA-BELLIDO 1986; STRUHL 1981; STRUHL and AKAM 1985; DUNCAN 1982; SATO, RUSSELL and DENELL 1983; DURA, BROCK and SANTAMARIA 1985; JÜRGENS 1985). Loss-of-function mutations in genes of the *Pc* group result in the ectopic expression of the *Ubx* gene in the T2 segment.

Loss-of-function mutants of the *Ubx* gene include *Ubx* alleles, which alter the protein coding portion of the gene (the Ubx transcription unit, see Figure 1), and the *abx*, *bx*, *pbx* and *bxd* groups of recessive alleles. These are located within the introns of the Ubx unit or upstream the Ubx unit; they presumably affect diverse regulatory regions (BENDER *et al.* 1983, 1985; BEACHY, HELFAND and HOGNESS 1985; HOGNESS *et al.* 1985; AKAM *et al.* 1985; WEINZIERL *et al.* 1987; LIPSHITZ, PEATTIE and HOGNESS 1987; O'CONNOR *et*

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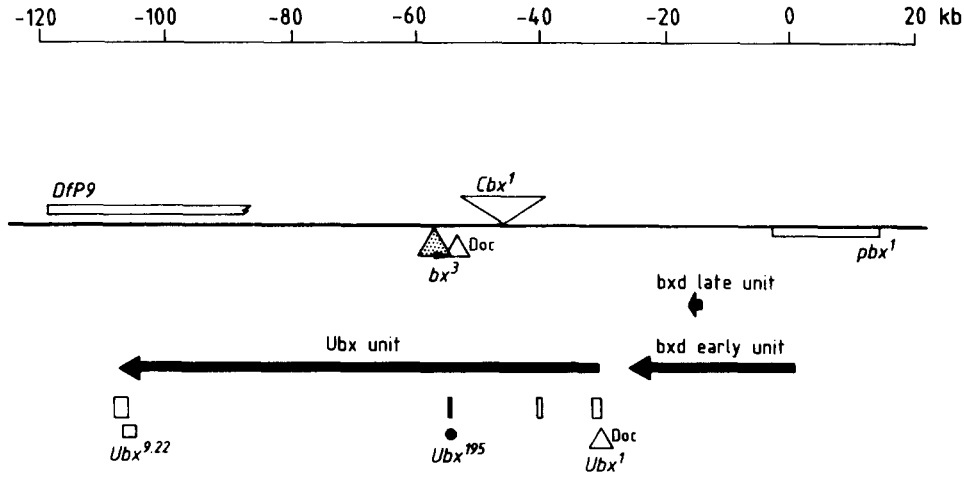

FIGURE 1.—Molecular map of the *Ubx* gene showing mutations used in this work. Rectangles indicate deletions and triangles insertions. Transcription units are indicated as heavy horizontal arrows, showing exons as rectangles. The dotted triangle is an insertion of the gypsy transposon, the arrow indicating the direction of its transcription. bx^3 is associated with two adjacent transposon insertions (gypsy and Doc), the second being irrelevant to the mutant phenotype. Cbx^1 corresponds to the insertion, in inverted orientation, of the DNA segment deleted in pbx^1. *Df(3R)P9* deletes the entire bithorax complex, its left end appearing in the drawing. Ubx^1 corresponds to the insertion of the Doc transposon in the 5′ untranslated region of the first Ubx unit exon, Ubx^{195} to a single nucleotide change introducing a nonsense codon in the exon at −53 kb, and $Ubx^{9.22}$ to a 1.5-kb deletion including the homeobox. All data for this figure were obtained from BENDER *et al.* 1983, 1985; PEIFER and BENDER 1986; WEINZIERL *et al.* 1987.

al. 1988). Gain-of-function mutants of the *Ubx* gene are generically termed *Contrabithorax* (*Cbx*) alleles (LEWIS 1978, 1982; CASANOVA, SÁNCHEZ-HERRERO and MORATA 1985; WHITE and AKAM 1985; MICOL and GARCÍA-BELLIDO 1988; GONZALEZ-GAITAN, MICOL and GARCÍA-BELLIDO 1990). They are associated with ectopic expression of the gene in T2 regions in which the wild-type allele is inactive. The best studied of those gain-of-function alleles is Cbx^1 which arose as a transposition of a 17-kb DNA segment from the upstream region to the second intron of the Ubx transcription unit (LEWIS 1982; BENDER et al. 1983; CASANOVA, SÁNCHEZ-HERRERO and MORATA 1985) (Figure 1).

Combinations of some mutant alleles of the *Ubx* gene show transvection, or synapsis-dependent complementation, as first described by LEWIS (1954) for that gene and later observed for other Drosophila loci (reviewed in JUDD 1988; WU and GOLDBERG 1989). In transvection phenomena the phenotype of a *trans* heterozygote between two different alleles of a gene changes depending upon whether the homologous alleles are paired. Although several hypotheses have been proposed, little is known about the biological meaning and the molecular basis of transvection effects.

In this paper we examine variations in the ectopic expression of the *Ubx* gene in the T2 segment, variations revealed both by the pattern of UBX in larval wing discs and by the morphologies of the adult wing structures. These variations were studied in genotypes involving double mutant chromosomes carrying in *cis* the gain-of-function Cbx^1 mutation and loss-of-function *Ubx* mutations which prevent the production of normal UBX. Expression was studied under different conditions of pairing between homologous chromosomes and in the presence or absence of the Pc^3 allele. These studies were carried out in the wing disc where the *Ubx* gene is normally not expressed and where any ectopic expression of the *Ubx* gene should be obvious. The results obtained provide evidence (1) of transvection visualized at the level of protein expression, (2) that the Cbx^1 mutant allele "induces" ectopic expression of the Ubx unit of a normal homolog, (3) that this phenomenon is not mediated by UBX, and (4) of the increased efficiency of this induction when levels of Pc^+ product are reduced. The term "induction" will be used throughout this paper without specific molecular implications, just to formally describe the activation of a Ubx^+ allele by a Cbx^1 alelle in *trans*.

MATERIALS AND METHODS

Fly stocks and culture: Flies were cultured on standard medium under uncrowded conditions at 25 ± 1°. All genetic variants used in this work have been previously described [LINDSLEY and GRELL (1968) and other references in the text].

Immunofluorescence staining: Larvae were obtained, dissected and stained with FP3.38 antibody as described in CABRERA, BOTAS and GARCÍA-BELLIDO 1985. At least 20 imaginal discs of each genotype were studied.

RESULTS

Adult wing phenotypes suggest that the Cbx^1 mutant allele induces ectopic expression of a paired

TABLE 1

Adult wing phenotypes and UBX wing disc patterns in genotypes used in this work

Genotypes		Pc^+		Pc^3	
		Adult wing phenotype	UBX pattern in wing discs	Adult wing phenotype	UBX pattern in wing discs
1	*+/Df(3R)P9*	A	a	A	a
2	*+/+*	A	a	B	b
3	*Dp(3;1)P115;+/Df(3R)P9*	A	a	B	b
4	*Cbx^1/+*	D	d	E	e
5	*Cbx^1Ubx^1/+*	C	c	D–E	d–e
6	*$Cbx^1Ubx^{9.22}$/+*	C	d	D–E	d–e
7	*Dp(3;1)P115;Cbx^1/Df(3R)P9*	D	d	E	e
8	*Dp(3;1)P115;Cbx^1Ubx^1/Df(3R)P9*	A	a	C	c
9	*Dp(3;1)P115;$Cbx^1Ubx^{9.22}$/Df(3R)P9*	A	d	C	d–e
10	*Dp(3;1)P115;Cbx^1Ubx^1/$Ubx^{9.22}$*	A	c	C	d–e
11	*Dp(3;1)P115;Cbx^1Ubx^1/Ubx^{195}*	A	c	C	d–e
12	*Ubx^1/+*	A	a	A	a
13	*$Ubx^{9.22}$/+*	A	a	A	b
14	*Ubx^{195}/+*	A	a	A	b
15	*Dp(3;1)P115;Ubx^1/Df(3R)P9*	A	ND	A	ND
16	*Dp(3;1)P115;$Ubx^{9.22}$/Df(3R)P9*	A	ND	A	ND

Wing phenotypic classes (A to E) and UBX patterns in discs (a to e) are defined in Figure 2. ND indicates not determined. *Dp(3;1)P115* is a translocation [*Tp(3;1)20;89B7-8;89E7-8*] of the BX-C from chromosome 3 to the heterochromatin of the *X* chromosome (LINDSLEY and GRELL 1968). *Df(3R)P9* is a deficiency (89D9-E1;89E4-5) for the entire BX-C (LINDSLEY and GRELL 1968). We have used the *Dp(3;1)P115;Df(3R)P9* combination to study genotypes in which the *Ubx* gene is located on another chromsome. Similar results were obtained using *Tp(3;3)P146*, in which the BX-C is located in the 3L chromosomal arm [*Tp(3;3)64C-E;89D1-2;90D1*] (LINDSLEY and GRELL 1968). It has been previously reported that the *Cbx^1*/+ mutant wing phenotype is slightly stronger than that of *Cbx^1/Df(3R)P9* (CASANOVA, SÁNCHEZ-HERRERO and MORATA 1985). This small difference, which can be interpreted in terms of induction of the normal chromosome by *Cbx^1*, is not considered here, both phenotypes being classified as class D.

normal *Ubx* gene: Table 1 gives a summary of the results obtained, on the basis of phenotypical classes defined as represented in Figure 2. The *Cbx^1* mutation causes homeotic transformations of the wing toward haltere, transformations which can be visualized both in the wing imaginal disc and in the adult wing (Figure 2, D and d). The extent of this transformation can be modified by the presence of different *cis*-associated *Ubx* mutant alleles and by the degree to which homologous *Ubx* gene regions are somatically paired. We have employed *Ubx* mutant alleles which are themselves morphogenetically inactive since they either lack detectable protein products (*Ubx^1*) or produce FP3.38-immunoreactive but truncated products lacking the homeodomain (*$Ubx^{9.22}$*, *Ubx^{195}*) (WEINZIERL *et al.* 1987; see Figure 1).

We will first consider experiments performed in a *Pc^+* genetic background. In *cis*-combinations with *Cbx^1*, both *Ubx^1* and *$Ubx^{9.22}$* mutations revert the Cbx adult wing phenotype (compare Figure 3, d with f), though not completely (compare genotype 4 with 5 and 6 in Table 1 and Figure 2, C with D). It has been proposed for *Cbx^1Ubx^1*/+ that the remnant mutant wing phenotype is due to ectopic activity of the normal *Ubx* homolog, induced by the *Cbx^1Ubx* chromosome if pairing between homologous Ubx chromosomal regions is not disturbed (the phenomenon designated "transvection;" LEWIS 1985). In order to test this hypothesis we have studied the same genetic combinations in structural heterozygotes that have the wild type allele of *Ubx* translocated to another chromosome (genotypes 8 and 9), a condition in which homologous BX-C regions are expected to be unpaired. In these combinations the Cbx phenotypes are further reduced compared to their controls (genotype 5 and 6 respectively), yielding an entirely wild-type wing. This finding supports E. B. LEWIS' inference that some *Cbx^1Ubx* chromosomes are capable of inducing the transcription of the *Ubx* homologous gene, only when both are paired; but cannot do it if the *Ubx* wild-type allele is in a translocated *Dp(3;1)P115* chromosome segment (genotypes 8 and 9). The proposed induction of the wild-type *Ubx* gene by a *Cbx^1Ubx* chromosome is confirmed by the wild-type phenotype of the adult wing when the homologous *Ubx* gene is also mutant and cannot produce functional UBX (genotypes 10 and 11).

Visualization of UBX expression patterns in wing discs demonstrates that the *Cbx^1* mutant allele induces ectopic expression of a paired normal *Ubx* homologous gene: Analysis of the UBX pattern of expression in the same combinations shows a major difference between *Cbx^1Ubx^1* and *$Cbx^1Ubx^{9.22}$ cis* double mutants. The patterns are consistent with the expectation (WEINZIERL *et al.* 1987) that the *Ubx^1* allele does not produce immunoreactive product while *$Ubx^{9.22}$* does. Thus, in genotype 8, for example, the wild-type UBX pattern reflects the behavior of the normal, unpaired *Ubx* allele [*Dp(3;1)P115*], which remains inactive. By contrast, we infer that the aberrant

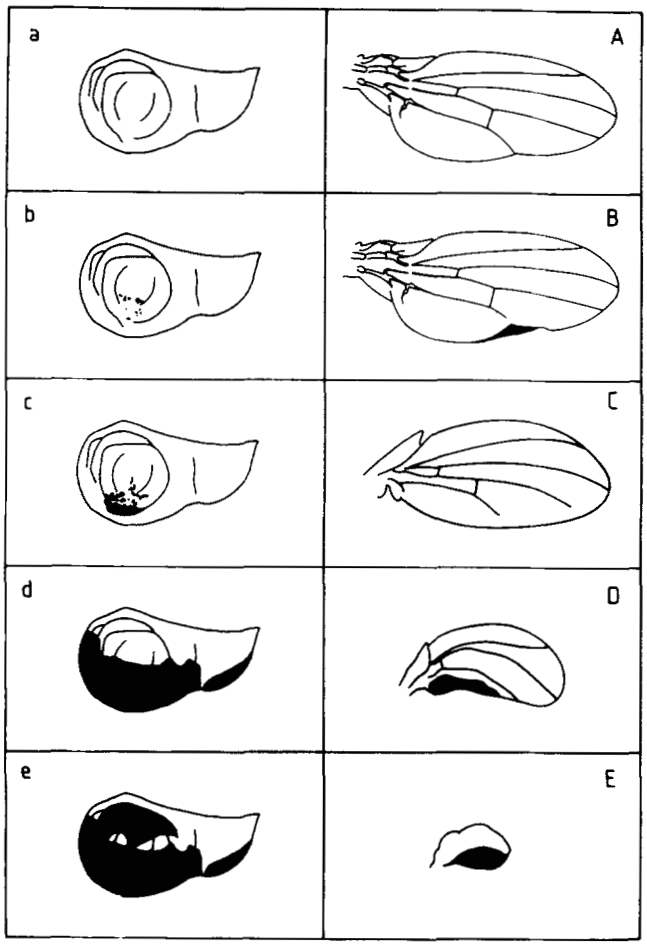

FIGURE 2.—Schematic representation of UBX wing disc patterns (a to e) and adult wing phenotypes (A to E) defining classes used in Table 1. Shaded areas in discs indicate positive immunofluorescence labelling against UBX. Mutant transformations toward haltere are shown in wings by the presence of haltere-like cells (shaded areas in B, D and E), and by the absence of wing structural elements such as the allula or some vein segments (in B and C). These are accompanied by reductions of wing surface (as B, C, D and E show). Although we have observed variations in phenotype among individuals of a given genotype, classes defined in the drawing do not overlap.

UBX pattern of genotype 9 reflects the superposition of the wild-type pattern with that of the immunoreactive protein products of $Ubx^{9.22}$ allele, derepressed by Cbx^1. That this pattern is quite similar to that of the single mutant chromosome Cbx^1 (genotype 7) suggests that the presence of the $Ubx^{9.22}$ mutation in *cis* to Cbx^1 does not alter the spatial specificity of that mutation. Immunofluorescence patterns confirm that Cbx^1Ubx^1 is able to induce the synthesis of UBX by its normal homolog only when they are paired (compare genotypes 5 and 8; Figure 3b). This interpretation is verified in genotypes 10 and 11 where the UBX products present in discs, although not contributing to the adult wing phenotype, must derive from the $Ubx^{9.22}$ and Ubx^{195} chromosomes. The adult wing phenotype of genotype 6 suggests that the $Cbx^1Ubx^{9.22}$ chromosome, like Cbx^1Ubx^1, is able to induce expression of a paired, normal homolog. However, this cannot be directly ascertained in wing discs because the $Ubx^{9.22}$ proteins show a pattern similar to that observed in Cbx^1 discs, which is superimposed to that of the normal UBX arising from the homolog (Figure 3, a and d).

The Pc^3 mutation enhances the induction: The presence in the genome of the Pc^3 mutation in heterozygotes causes a weak derepression of the wild-type *Ubx* gene in T2, visualized both by immunofluorescence in wing discs and by the adult phenotype (Figure 2, B and b). This mutant phenotype (1) is dependent on the number of doses of the *Ubx* gene, being virtually wild type in the presence of only one normal dose of *Ubx* but becoming stronger with increasing doses (CAPDEVILA, BOTAS and GARCÍA-BELLIDO 1986; BOTAS, CABRERA and GARCÍA-BELLIDO 1988) (compare genotypes 1 and 2 in Table 1), and (2) is not dependent on the location in the genome of copies of the *Ubx* gene (compare genotypes 2 and 3 in Table 1). The Polycomb adult wing phenotype disappears in all heterozygotes between the wild-type allele and a *Ubx* mutation, but not so the aberrant UBX wing disc pattern (compare genotype 1 with 12, 13 and 14). This pattern is retained in $Ubx^{9.22}$ and Ubx^{195} heterozygotes, showing that these mutations (and possibly Ubx^1 as well) do not affect the regulatory element(s) controlled by Pc^+. The Pc^3Cbx^1 combination shows an extreme Cbx phenotype in both paired and unpaired conditions (genotypes 4 and 7). Pairing effects can be appreciated, however, in Pc^3 and Cbx^1Ubx^1 or $Cbx^1Ubx^{9.22}$ combinations (compare genotypes 5 with 8 and 6 with 9). In the former case the lack of homologous pairing reduces both the adult wing phenotype and imaginal disc UBX expression and in the latter (as expected) only the adult wing phenotype. Interestingly in $Pc^3Cbx^1Ubx/+$ combinations both the pattern of UBX expression in the wing disc and the adult wing phenotype are similar to those obtained in $Cbx^1/+$ individuals. This indicates that the spatial pattern of ectopic expression of the normal *Ubx* allele in these experiments is imposed by the Cbx^1 chromosome, at a low level in a Pc^+ background and at a high level in a Pc^3 one. Individuals with genotypes 10 and 11, in which the putatively induced chromosomes yield nonfunctional UBX, show Cbx wing disc UBX patterns, but nearly wild-type adult wing phenotypes. These observations support again the idea that Cbx^1 is imposing its own spatial pattern of expression to the homolog.

Remarkably, the enhancement by Pc^3 of the induction phenomenon is observable even when the homologs are unpaired. As seen in genotypes 8 to 11, the Cbx^1Ubx chromosomes, which are unable to induce ectopic activity of the translocated *Ubx* gene in a Pc^+ background, are able to do so (albeit to a low extent) in the presence of the Pc^3 mutation. This effect is not explicable as a direct effect of decreased repression by *Pc* on the translocated *Ubx* gene: comparison of

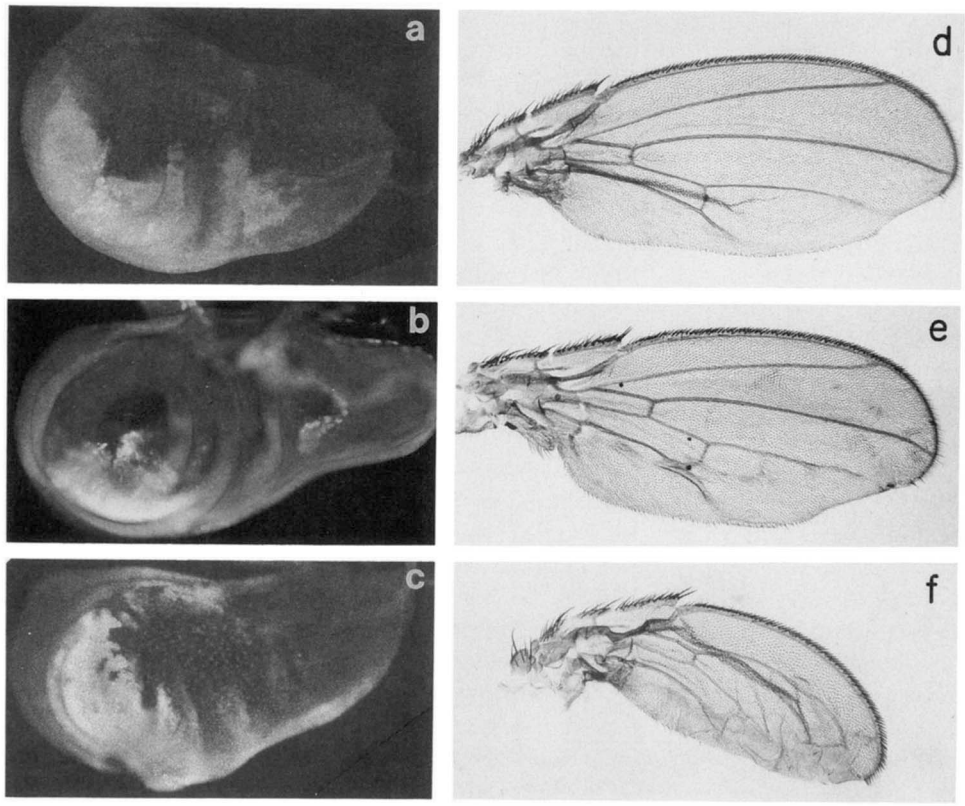

FIGURE 3.—Representative examples of UBX wing disc patterns and wing phenotypes of genotypes studied in this work. (a) Anti-UBX immunofluorescence staining by FP3.38 antibody of a $Cbx^1Ubx^{9.22}$/+ wing disc, showing positive signal in most posterior compartment cells. This UBX pattern is classified as d in Figure 2. (b) UBX pattern in a Cbx^1Ubx^1/+ wing disc, which corresponds to class c in Figure 2. This pattern is quite similar to that found in $Dp(3;1)P115;Pc^3Cbx^1Ubx^1/Df(3R)P9$. (c) UBX pattern in a $Pc^3Cbx^1Ubx^1$/+ wing disc, which corresponds to class e in Figure 2. Signal is observed in all posterior and some anterior compartment cells. (d) Adult wing phenotype of a $Cbx^1Ubx^{9.22}$/+ individual, which corresponds to class C in Figure 2. The mutant phenotype is identical to the observed in Cbx^1Ubx^1/+ flies, despite their different UBX wing disc patterns (compare Figure 3a with 3b). (e) Adult wing phenotype of a $Dp(3;1)P115;Pc^3Cbx^1Ubx^1/Df(3R)P9$ individual. The mutant transformation is similar to that shown in d. (f) Adult wing phenotype of a $Pc^3Cbx^1Ubx^1$/+ individual, which is classified as class D in Figure 2. This phenotype is similar to that of Cbx^1/+ individuals.

genotypes 8 to 11 with those of 15 and 16 (see column of adult phenotypes in the presence of Pc^3) show that Cbx^1 is required to obtain an appreciable activation of the $Dp(3;1)P115$. This is even clearer in the comparison of genotype 3, having two normal *Ubx* genes derepressed by Pc^3 but showing little adult mutant phenotype, with genotypes 8 to 11, which have only one normal *Ubx* gene able to contribute to the mutant phenotype.

***Ubx*^{195} and *Ubx*$^{9.22}$ alleles produce normal UBX patterns in the haltere disc:** The results described above were obtained by studying ectopic expression of the *Ubx* gene in the T2 segment. In addition, we have studied the behavior of Ubx^1, Ubx^{195} and $Ubx^{9.22}$ alleles in T3, the normal realm of action of the gene, in the absence of Cbx^1 mutation and in a Pc^+ background. Ubx^1 heterozygotes with strong loss-of-function recessive mutations (bx^3 and pbx^1; see Figure 1) show mutant phenotypes in the adult haltere (homeotic transformations towards wing) which are associated to the absence of UBX in the corresponding presumptive regions of the haltere disc. Similar wing phenotypes were observed in heterozygotes involving the same recessives and $Ubx^{9.22}$ or Ubx^{195} mutations. The UBX haltere disc patterns were in these cases almost wild-type. Therefore, $Ubx^{9.22}$ and Ubx^{195} mutations do not alter the spatial pattern of expression of the *Ubx* gene in T3.

DISCUSSION

A mutant allele induces in *trans* the ectopic activity of a normal homolog: Transvection has been defined as complementation dependent on pairing between homologous alleles [LEWIS (1954); reviewed in JUDD (1988) and WU and GOLDBERG (1989)]. In the vast majority of cases mutations involved in transvection effects are loss-of-function alleles and complementation is decreased if pairing is disrupted. Most of the studies of transvection effects have been largely inferential, given that only adult mutant phenotypes were considered. We show here a case in which a gain-of-function mutation (Cbx^1) imposes aberrant behavior (ectopic expression in T2) upon a normal allele of the gene located in the homologous chromosome. This has been studied under conditions in which putative inducer and induced gene activities can be distinguished because (1) the mutant Cbx^1 allele carries a second mutation which prevents the production of morphogenetically active UBX, and (2) the normal *Ubx* allele is inactive in the tissues under study. Induction was inferred from the adult wing phenotype and demonstrated by visualization of UBX in wing discs, thus providing evidence of transvection in the *Ubx* gene at the level of protein synthesis, and corroborating a previous hypothesis of E. B. LEWIS, which was supported only by observations on mutant phenotypes.

The induction phenomena studied here are not mediated by UBX since (1) the inducer chromosomes lack UBX (*Cbx*^{1}*Ubx*^{1}) or produce UBX lacking the part of the molecule including the homeodomain (*Cbx*^{1}*Ubx*$^{9.22}$), and (2) the induced chromosomes show the induction effect irrespectively of being able to yield normal UBX, *i.e.*: the *Ubx*$^{9.22}$ and *Ubx*^{195} single mutant chromosomes are induced in the same way in which the wild-type one is. In addition, it should be noted that a phenomenon dependent upon pairing between alleles located in homologous chromosomes can not easily be explained in terms of the protein products of the interacting alleles.

Although all the induction effects presented here involve *Cbx*^{1} mutation, transvection in T2 cells is not an exclusive property of the *Cbx*^{1} allele. Spontaneous revertants of *Cbx*^{1} (*Cbx*^{1RM}) and *Cbx*^{2} (*Cbx*^{2RM}) are known to be involved in transvection effects (MICOL and GARCÍA-BELLIDO 1988) and one revertant of *Cbx*^{M1} (*Cbx*^{M1RM}) has been obtained which, in the presence of *Pc*^{3}, is able to induce ectopic expression of a normal homolog (J. E. CASTELLI-GAIR, unpublished results). However, on the basis of our present results it is not possible to determine if induction phenomena involving *Cbx* mutations evidence, by ectopic derepression in T2, functions normally involved in the regulation of the gene in T3 or if they are merely a consequence of the aberrant behavior of *Cbx* mutations.

Implications for transvection models: There is increasing evidence of regulation of transcription by interactions between proteins bound to separate DNA sites (PTASHNE 1986, 1988). Such action at a distance in DNA has been proposed to be possible not only between different regions of a gene, but also between two homologous alleles if they are paired (BENSON and PIRROTTA 1988). Interactions between some activated regulatory element in *Cbx*^{1} and the promoter of the normal homolog could be taken as an explanation for the transvection effects presented in this work. However, we find difficult to explain under that hypothesis the induction by *Cbx*^{1} of both the paired and the translocated alleles, as observed in the presence of *Pc*^{3} mutation (genotypes 8 to 11). Pairing between the activated regulatory element of *Cbx*^{1} and the two promoters should be assumed, requiring explanation the differential effectiveness of the induction effect on the promoter of the paired allele (which is induced at a low level in a *Pc*$^{+}$ background and induced at a high level in a *Pc*^{3} background) and on the promoter of the translocated, unpaired allele (which is not induced in a *Pc*$^{+}$ background but induced at a low level in a *Pc*^{3} background).

As an alternative hypothesis to explain transvection, some have suggested the involvement of diffusible transcriptional factors, with differential distribution inside the cellular nucleus (LEWIS 1985; KORNHER and BRUTLAG 1986). Some authors have speculated that these transcription factors could be short-radius-of-action regulatory RNAs transcribed from the genes involved in the transvection phenomenon (JACK and JUDD 1979; MICOL and GARCÍA-BELLIDO 1988; MICOL, CASTELLI-GAIR and GARCÍA-BELLIDO 1990; D. MATHOG, personal communication). If Ubx RNAs of short radius of action were the inductive signals produced by a *Cbx*^{1} chromosome, they would be able ectopically to activate a paired *Ubx* normal gene. If their production were increased, or their reception favoured as an effect of the *Pc*^{3} mutation they would be able to activate also the distant, nonpaired copy of the *Ubx* gene.

The role of *Polycomb* in transvection: The inducer *Cbx*^{1} chromosome has a UBX pattern in wing discs (see genotypes 7 and 9, in a *Pc*$^{+}$ background; the pattern is essentially the same that the one shown in Figure 3a) which is different from that observed for the induced chromosome (see Figure 3b and genotypes 5, 10, and 11, in a *Pc*$^{+}$ background): the induced chromosome produces detectable UBX only in a subset of the cells in which the inducer chromosome does so. The different spatial patterns of inducer and induced gene activities suggest that transvection occurs only in a subset of the cells where *Cbx*^{1} is expressed. This observation might be explained (1) by differential distribution in the cells of the anlage of inductive abilities of *Cbx*^{1}, being gene expression and inductive competence noncorrelated properties, or (2) by differential distribution of receptiveness to induction by the homologous allele. When the *Pc*^{3} mutation is also present in the genome, transvection becomes detectable (both in UBX disc patterns and adult phenotype) in all the cells in which *Cbx*^{1} is active. Since *Pc*$^{+}$ products are supposed to be transcriptional repressors of *Ubx* gene activity, these results could be interpreted as an enhancement of the transcription of the inductive signal, arising from the *Cbx*^{1}*Ubx* chromosomes and reaching the homologs and/or to an increase in the receptiveness in the induced chromosome.

Our results show that *Pc*$^{+}$ products act preventing *Cbx*^{1} dependent transvection to occur in a large number of wing disc cells. Analogous results have been obtained in studies of the strong mutant phenotype of some heterozygotes involving the loss-of-function *pbx*^{1} mutation, which is rescued by *Pc*^{3} (CAPDEVILA, BOTAS and GARCÍA-BELLIDO 1986; J. E. CASTELLI-GAIR and A. GARCÍA-BELLIDO, manuscript in preparation) and by *super sex combs* (a *Polycomb*-like mutation; INGHAM 1984). It is interesting to note that the same DNA segment absent in the *pbx*^{1} deletion is ectopically inserted in the Ubx transcription unit in *Cbx*^{1} mutant (Figure 1). However, the *Pc*^{3} mutation interacts similarly with *Cbx*^{M1RM}, which is, as far as we know, not related to *pbx*^{1}. In spite of these observations, we think it unlikely that one of the normal roles

of Pc^+ during development is to prevent transvection. It has been shown that *Pc* gene products repress other selector genes in cells where *Ubx* gene is active (WEDEEN, HARDING and LEVINE 1986). Therefore, we think it more likely that the inductive signal due to Cbx^1 in T2 is associated with positive autoregulation of the *Ubx* gene in T3. In this light, transvection involving *Cbx* alleles would be interpreted as the accidental activation of a normal allele by an autoregulatory mechanism which is ectopically active in its homolog.

Implications for autoregulation: Genetic studies led to the proposal that the BX-C genes regulate the activities of other genes (LEWIS 1964; GARCÍA-BELLIDO 1975). On the basis of developmental and genetic studies, we have also inferred a role for the *Ubx* gene products in maintaining the activity of the gene itself (GARCÍA-BELLIDO and CAPDEVILA 1978; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; CAPDEVILA, BOTAS and GARCÍA-BELLIDO 1986; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). Recent studies show that several homeotic genes of Drosophila are subject to autoregulation (reviewed in SERFLING 1989). However, little is known about the molecular nature of this putative positive autoregulation in the case of the *Ubx* gene. It has been shown recently (1) that mutational damages affecting UBX structure reduce, but do not abolish, *Ubx* gene positive autoregulation in the embryonic visceral mesoderm (BIENZ and TREMML 1988), where the gene seems to be under different regulatory controls than in other germ layers (BIENZ *et al.* 1988), (2) that one of the UBX binds sequences near the promoter of the *Ubx* gene (BEACHY *et al.* 1988), and (3) that UBX positively autoregulate the *Ubx* gene promoter in cultured Drosophila cells (KRASNOW *et al.* 1989). Despite the observations (2) and (3), it has been suggested that positive autoregulation of the *Ubx* gene is restricted *in vivo* to the visceral mesoderm (KRASNOW *et al.* 1989).

Both $Ubx^{9.22}$ and Ubx^{195} alleles produce morphogenetically inactive UBX lacking the homeodomain. In spite of that, they (1) are able to give a wild-type UBX spatial pattern in T3, (2) do not modify in T2 the UBX pattern of Cbx^1 when arranged in *cis* with this mutation, and (3) behave like the wild-type *Ubx* allele in their responses to induction by a Cbx^1 allele in *trans*. We think that these observations are compatible either with (1) the unexistence of positive autoregulation of the *Ubx* gene in imaginal discs, or (2) the existence of a mechanism of autoregulation not requiring the homeodomain of UBX.

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